^{14}C-LABELING OF A NOVEL FUNGICIDE. I. SYNTHESES OF OPTICALLY ACTIVE (E)- AND (Z)-1-(2,4-DICHLOROPHENYL)-4,4-DIMETHYL-2-(1,2,4-TRIAZOL-[^{14}C]-1-YL)-1-PENTEN-3-OLS

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SUMMARY

A novel fungicide, (-)(E)-1-(2,4-dichlorophenyl)-4,4-dimethyl-2-(1,2,4-triazol-1-yl)-1-penten-3-ol (S-3308 L) and its three optically active stereoisomers were labeled with carbon-14 at the triazole ring for use in the metabolic and environmental fate studies. 1,2,4-Triazole-^{14}C (4) prepared from formamide-^{14}C (3) was treated with bromopinacolone to give triazolylpinacolone-^{14}C (5), which was condensed with 2,4-dichlorobenzaldehyde giving (Z)-ketone-^{14}C (6). (Z)-Ketone-^{14}C (6) was photoisomerized to (E)-ketone-^{14}C (7), which was reduced to racemic (E)-S-3308-^{14}C (1) in 20% yield from 3. Reduction of 6 gave racemic (Z)-S-3308-^{14}C (2) in 23% yield from 3. Optical resolution of both racemates 1 and 2 by a HPLC method with a chiral column gave (-)(E)-, (+)(E)-, (-)(Z)- and (+)(Z)-1-(2,4-dichlorophenyl)-4,4-dimethyl-2-(1,2,4-triazol-[^{14}C]-1-yl)-1-penten-3-ols (1a, 1b, 2a and 2b) in essentially quantitative yields.

Key Words: Carbon-14, Optically Active, Fungicide, 1,2,4-Triazole-^{14}C

INTRODUCTION

A novel triazole fungicide, S-3308 L, (-)(E)-1-(2,4-dichlorophenyl)-4,4-dimethyl-2-(1,2,4-triazol-1-yl)-1-penten-3-ol ((-)(E)-S-3308) has been found to possess potent toxicity against a broad range of fungal species, especially against those belonging to *Ascomycetes* and *Basidiomycetes*[1,2]. There are three possible stereoisomers of S-3308 L such as (+)(E)-, (-)(Z)- and (+)(Z)-1-(2,4-dichlorophenyl)-4,4-dimethyl-2-(1,2,4-triazol-1-yl)-1-penten-3-ols, which are designated (+)(E)-, (-)(Z)- and (+)(Z)-S-3308, respectively,

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with regard to asymmetry at C-3 and the substituent at C-1.

In order to compare the metabolism of S-3308 L with its stereoisomers in mammals, insects, plants, fishes and so on, it was necessary to prepare radioactive ones. In this paper, we describe the synthetic methods for ^{14}C-labeling of S-3308 L ((-)(E)-S-3308) and its three stereoisomers ((+)(E)-, (-)(Z)- and (+)(Z)-S-3308) at the triazole ring.

DISCUSSION

Figure 1 illustrates the procedure for the syntheses of (-)(E)-, (+)(E)-, (-)(Z)- and (+)(Z)-S-3308-(triazole-^{14}C). This work has been achieved by overcoming the following major problems: 1) preparation of 1,2,4-triazole-^{14}C; 2) isomerization of (Z)-ketone-^{14}C to (E)-ketone-^{14}C; and 3) optical resolution of (E)- and (Z)-S-3308-(triazole-^{14}C).

There has been no report concerning the preparation of 1,2,4-triazole-^{14}C so far although several methods for the synthesis of the non-radioactive compound have been published[4-8]. Ainsworth *et al.* reported the synthesis of 1,2,4-triazole, which involved the preparation of N,N-diformylhydrazine from hydrazine and formic acid or formamide, followed by treatment of N,N-diformylhydrazine with an excess amount of liquid ammonia[6]. In our small scale preparation, N,N-diformylhydrazine was obtained in a good yield but the subsequent cyclization with liquid ammonia gave only a very low yield of 1,2,4-triazole. After considerable trials, we found a new useful method for the radioactive preparation. Thus, formamide-^{14}C (3) was treated with an equivalent amount of freshly prepared N,N-diformylhydrazine at 160-170 °C for 9 hr to give 1,2,4-triazole-^{14}C (4) in a moderate yield. Since the isolation of 4 leads to loss of yield, the crude product was used for the following step without any purification. Treatment of 4 with sodium ethoxide followed by bromopinacolone gave triazolylpinacolone-^{14}C (5) in 31% yield from 3 after purification by a column chromatography on silica gel.

The specific activity of 5 was found to be about 2/3 as much as that of formamide-^{14}C used. The mechanism of 1,2,4-triazole formation has not been clarified yet, but this finding can be explained by supposing the following

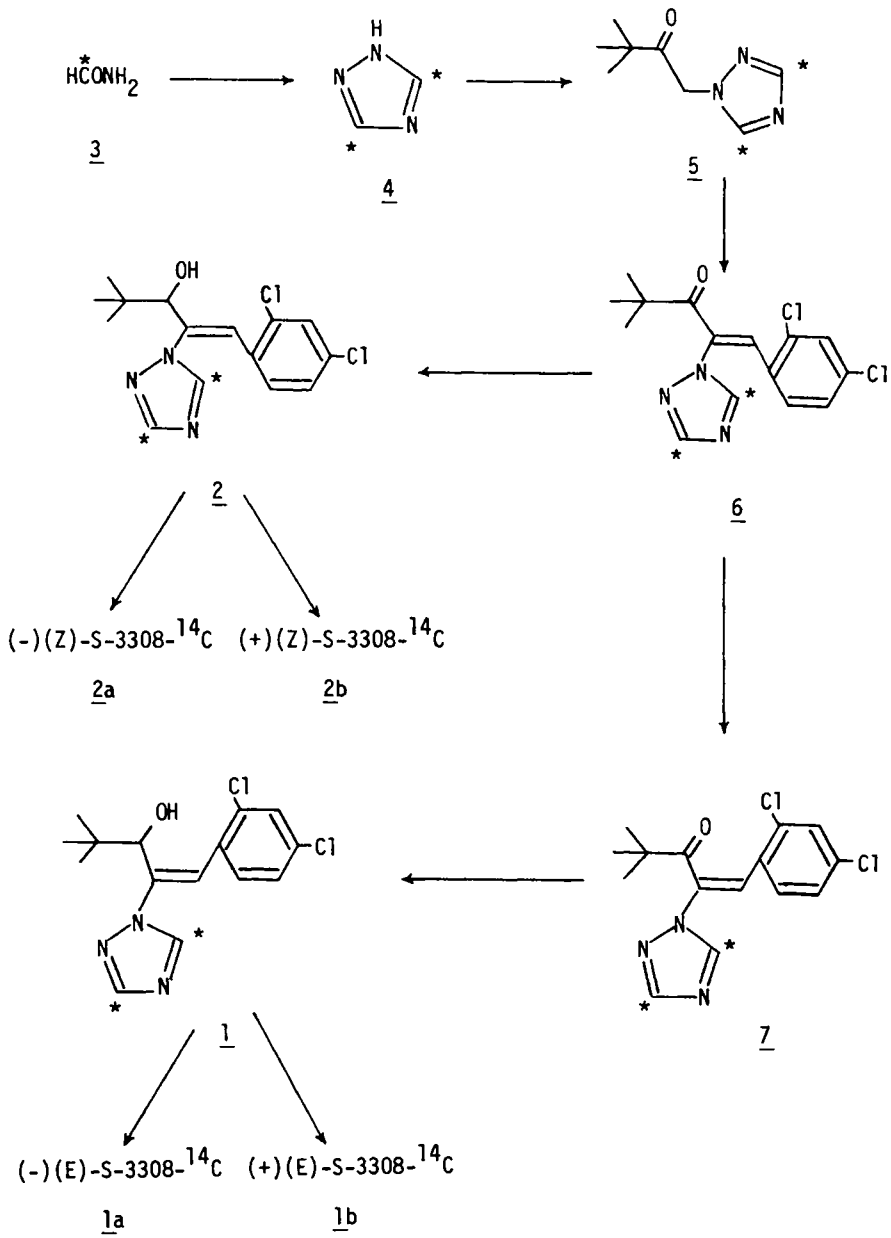

Fig. 1 Scheme for the synthesis of (-)(E)-, (+)(E)-, (-)(Z)- and (+)(Z)-S-3308-(triazole-^{14}C)

process: 1) the exchange of the formyl groups between formamide-^{14}C (3) and N,N-diformylhydrazine (8) might take place prior to the cyclization of a postulated intermediate (9) to give radioactive N,N-diformylhydrazine (8') (about 2/3 specific activity of formamide-^{14}C used) and diluted formamide-^{14}C

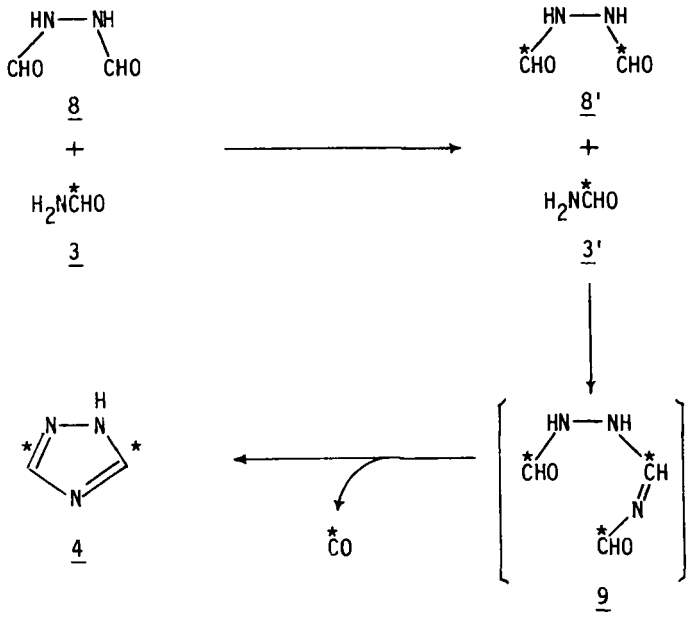

Fig. 2. A possible mechanism for the formation of 1,2,4-triazole-^{14}C

(3')(about 1/3 specific activity of original one); and 2) radioactive N,N-diformylhydrazine (8') might be cyclized to 1,2,4-triazole-^{14}C *via* the intermediate (9) as illustrated in Figure 2. This process is supported by the fact that radioactive N,N-diformylhydrazine (8') was detected in the reaction mixture of the triazole formation step.

Triazolylpinacolone-^{14}C (5) was allowed to react with 2,4-dichlorobenzaldehyde in the presence of triethylamine to afford a 95:5 mixture of (Z)- and (E)-ketone-^{14}C, which was purified by column chromatography on silica gel giving (Z)-ketone-^{14}C (6) in 84% yield. (Z)-ketone-^{14}C (6) was reduced with sodium borohydride in the presence of catalytic amount of sulfuric acid in methanol to give (Z)-S-3308-(triazole-^{14}C)(2) in 90% yield after purification by column chromatography on silica gel. In this reduction, a small amount of sulfuric acid was very efficient to prevent the formation of unfavorable by-products.

Photoisomerization of non-radioactive (Z)-ketone to (E)-ketone has been reported by Funaki *et al.*[3]. We successfully applied this method with some modifications suitable for the radioactive preparation. Thus, toluene was chosen as a solvent instead of acetone, used in the original method, to avoid

Table 1 Retention times of the four stereoisomers on chiral HPLC columns

Stereoisomers	Retention time (min)	Column & Condition
(-)(E)-S-3308	12.2	A
(+)(E)-S-3308	15.0	A
(-)(Z)-S-3308	18.5	B
(+)(Z)-S-3308	21.2	B

A: column: Sumipax OA-2200 (4 mm x 25 cm)
solvent: n-hexane/1,2-dichloroethane/ethanol = 350/40/4
flow rate: 1.0 ml/min
B: column: Sumipax OA-4200 (4 mm x 25 cm)
solvent: n-hexane/1,2-dichloroethane/ethanol = 700/70/5
flow rate: 0.8 ml/min

the unfavorable effects due to the decrease of the volume during the reaction. A solution of 6 in toluene was irradiated with uv light for 1 hr with bubbling nitrogen to give a 9:1 mixture of 7 and 6. Since it was rather difficult to remove 6, the resulting product was immediately reduced by the same manner used in the reduction of 6 to afford a 9:1 mixture of (E)- and (Z)-S-3308-(triazole-^{14}C)(1) in 78% yield from 6, together with 2 (9%).

Optical resolution of non-radioactive (E)- and (Z)-S-3308 has been carried out by the method using the diastereoisomeric ester[4]. This method, however, does not seem to be proper for the radioactive preparation because of its tedious process and low yields especially in a small scale preparation. Recently, Ohi *et al.* reported that the HPLC methods with chiral columns can resolve various enantiomers such as amino acids, amines and acids[9-11]. Expecting that this method could be applied for the resolution of the radioactive compounds, we examined the separation of the racemates 1 and 2 on the various chiral columns. As a result, we found that Sumipax OA-2200 column (chiral stationary phase: N-(1R, 3R)-*trans*-chrysanthemoyl-(R)-phenylglycyl-aminopropylsilica) and OA-4200 column (chiral stationary phase: N-(R)-1-naphthylethylamidocarbonyl-(R)-phenylglycineaminopropylsilica) were the best columns for 1 and 2, respectively, as shown in Table 1.

By the use of these columns, the stereoisomers, (-)(E)-, (+)(E)-, (-)(Z)- and (+)(Z)-S-3308-(triazole-^{14}C)(1a, 1b, 2a and 2b) were obtained in quantitative yields. These final products were identical in every respect with the unlabeled authentic samples.

EXPERIMENTAL

Thin-layer chromatography (TLC) was carried out on silica gel 60 F_{254} plate (Merck) with the following solvent systems: solvent A, chloroform/ethyl acetate/methanol/28% aqueous ammonia = 60/30/10/1; solvent B, chloroform/methanol = 4/1; solvent C, benzene/acetone = 1/1; solvent D, toluene/acetonitrile/methanol = 40/8/2; solvent E, benzene/ether = 8/2; solvent F, benzene/ether = 7/3; solvent G, acetonitrile/methanol = 9/1. Gas chromatography (GC) was conducted on a Yanaco GC-80 gaschromatograph (Yanagimoto Co., Ltd., Japan) equipped with a RD-4 gas-flow GM-counter (Nihon Musen Co., Ltd., Japan) with the following glass columns and conditions: GC_1, column: 2% FFAP on Uniport HP (3 mm x 3 m), column temperature: 170 °C, He: 30 ml/min; GC_2, column: 5% XE-60 on Chromosorb WAWDMCS (3 mm x 2 m), column temperature: 200 °C, He: 47 ml/min; GC_3, column: 2% FFAP on Uniport HP (3 mm x 3 m), column temperature: 260 °C, He: 60 ml/min. IR spectra were determined by a Jasco IR-810 (Nihon Buncoh Co., Ltd., Japan). NMR spectra were recorded on a Hitachi R-24 B (Hitachi Co., Ltd., Japan) with tetramethylsilane as an internal standard. High performance liquid chromatography (HPLC) was carried out on a Waters model 6000 liquid chromatograph equipped with Aloka radioanalizer RLC-551 (Aloka Co., Ltd., Japan). Formamide-^{14}C (170 mCi, specific acitivity (38.6 mCi/mmole)) was purchased from Amersham International plc (England). Chiral HPLC columns, Sumipax OA-2200 ^{R} and Sumipax OA-4200 ^{R} for analytical and preparative HPLC were purchased from Sumika Chemical Analysis Service Ltd. (Japan).

1,2,4-Triazole-^{14}C (4) -- A mixture of formamide-^{14}C (170 mCi, 198 mg, 4.40 mmole) and N,N-diformylhydrazine (388 mg, 4.40 mmole) freshly prepared by Ainsworth's method[6] was heated at 160-170 °C for 9 hr under nitrogen stream. Carbon monoxide formed during the reaction was trapped into a solution of copper chloride in 28% aqueous ammonia. After cooling, ethanol and benzene were added

to the mixture, and then the solvent was distilled under atmospheric pressure to give a residue, which was used for the next step without any purification.

1-(1,2,4-Triazol-[^{14}C]-1-yl)-3,3-dimethylbutane-2-one (5) -- A mixture of 1,2,4-triazole-^{14}C and a solution of sodium ethoxide (313 mg, 4.60 mmole) in ethanol (2.2 ml) was heated at 110-120 °C for 1 hr. After cooling, bromopinacolone (827 mg, 4.62 mmole) was added to the reaction mixture, and the mixture was stirred at 50-60 °C for 2 hr. After cooling, the mixture was diluted with water, extracted with chloroform, and the extract was washed with 5% sodium carbonate and water, dried, and evaporated to give a residue. Column chromatography of the residue on silica gel with chloroform gave 1-(1,2,4-triazol-[^{14}C]-1-yl)-3,3-dimethylbutane-2-one (5)(52.5 mCi, 341 mg, specific activity 25.7 mCi/mmole, yield 31%, purity 99%); mp 64 °C; TLC: Rf(A) 0.45, Rf(B) 0.60, Rf(C) 0.33; GC_1: retention time 9.0 min; IR νmax (chloroform): 1735 cm^{-1} (C=O): NMR (δ, $CDCl_3$): 1.30 (1H, s, tert-butyl H), 5.20 (2H, s, methylene H), 7.95 (1H, s, triazole H), 8.16 (1H, s, triazole H).

(Z)-1-(2,4-Dichlorophenyl)-4,4-dimethyl-2-(1,2,4-triazol-[^{14}C]-1-yl)-1-penten-3-one (6) -- A mixture of 1-(1,2,4-triazol-[^{14}C]-1-yl)-3,3-dimethylbutane-2-one (52.5 mCi, 341 mg, 2.04 mmole), triethylamine (2.04 g), 2,4-dichlorobenzaldehyde (1.07 g, 6.13 mmole) and acetic anhydride (4.2 ml) was stirred at 55-65 °C for 5 hr under nitrogen. After cooling, the mixture was diluted with water, made alkaline with sodium carbonate, and extracted with ether. The extract was washed with water, dried, and evaporated to give a residue, which was shown by TLC (solvent E) to be a 95:5 mixture of the (Z)- and (E)-isomers. The residue was purified by column chromatography with benzene-ether = 9/1 to afford (Z)-1-(2,4-dichlorophenyl)-4,4-dimethyl-2-(1,2,4-triazol-[^{14}C]-1-yl)-1-penten-3-one (6)(44.1 mCi, 558 mg, yield 84%, purity 99%); mp 120 °C; TLC: Rf(D) 0.51, Rf (E) 0.29; GC_2: retention time 15.5 min; IR νmax (chloroform): 1680 cm^{-1} (C=O); NMR (δ, $CDCl_3$): 1.30 (9H, s, tert-butyl H), 6.38-7.45 (1H, m, aromatic H), 7.52 (1H, s, olefinic H), 7.90 (1H, s, triazole H), 8.00 (1H, s, triazole H).

(±)(Z)-1-(2,4-Dichlorophenyl)-4,4-dimethyl-2-(1,2,4-triazol-[^{14}C]-1-yl)-1-penten-3-ol ((Z)-S-3308-(triazole-^{14}C))(2) -- A solution of sulfuric acid (0.9 mg) in methanol (90 µl) and sodium borohydride (55 mg, 1.46 mmole) were added to a stirred, cooled solution of (Z)-1-(2,4-dichlorophenyl)-4,4-dimethyl-2-(1,2,4-triazol-[^{14}C]-1-yl)-1-penten-3-one (6)(17.4 mCi, 219 mg, 0.677 mmole) in methanol (6.1 ml). The mixture was stirred at 0-5 °C for 1.5 hr. After decomposition with water, and hydrochloric acid, the mixture was extracted with ether. The extract was washed with water, dried, and evaporated to give a residue. Chromatography of the residue on silica gel with benzene-ether (gradient) gave (Z)-S-3308-(triazole-^{14}C)(2)(15.7 mCi, 199 mg, yield 90%, purity 99%); mp 162 °C; TLC: Rf(D) 0.45, Rf(F) 0.14, Rf(G) 0.55; GC_3: retention time 17.5 min; NMR (δ, $CDCl_3$): 0.82 (1H, s, tert-butyl H), 3.60 (1H, bs, hydroxyl H), 4.44 (1H, s, methine H), 6.40-7.50 (4H, m, aromatic and olefinic H), 7.80 (1H, s, triazole H), 8.00 (1H, s, triazole H).

Optical resolution of (Z)-S-3308-(triazole-^{14}C)(2) -- A solution of (Z)-S-3308-(triazole-^{14}C)(2)(15.7 mCi, 199 mg) in dioxane (995 µl) was injected by portions (50 µl) on a HPLC system (column: Sumipax OA-4200 , 8 mm x 25 cm (x 2), mobile phase: n-hexane/1,2-dichloroethane/ethanol = 700/70/5, flow rate: 4 ml/min). Fractions containing the (-)-isomer (retention time: 18.5 min) and the (+)-isomer (retention time: 21.5 min) were collected, and evaporated to give (-)(Z)-S-3308-(triazole-^{14}C)(2a)(7.6 mCi, 96 mg, specific activity 25.7 mCi/mmole, yield 99%, purity 99%) and (+)(Z)-S-3308-(triazole-^{14}C)(2b)(7.8 mCi, 99 mg, specific activity 25.7 mCi/mmole, yield 99%, purity 99%), respectively. These products were identical in every respect with the unlabeled authentic samples.

(E)-1-(2,4-Dichlorophenyl)-4,4-dimethyl-2-(1,2,4-triazol-[^{14}C]-1-yl)-1-penten-3-one (7) -- A solution of (Z)-1-(2,4-dichlorophenyl)-4,4-dimethyl-2-(1,2,4-triazol-[^{14}C]-1-yl)-1-penten-3-one (6)(26.7 mCi, 337 mg, 1.04 mmole) in toluene (17 ml) was irradiated with the light of a 500 W high pressure mercury lamp (Koeisha EHB-WI-500, Koeisha Ltd., Japan) for 1 hr with bubbling nitrogen.

The solvent was evaporated to give a residue (26.7 mCi), consisting of an approximately 9:1 mixture of 7 and 6. The product was used the following reaction without any purification.

(±)(E)-1-(2,4-Dichlorophenyl)-4,4-dimethyl-2-(1,2,4-triazol-[^{14}C]-1-yl)-1-penten-3-ol ((E)-S-3308-(triazole-^{14}C))(1) -- A solution of sulfuric acid (1.2 mg) in methanol (120 µl) and sodium borohydride (77 mg, 2.04 mmole) were added to a stirred, cooled solution of (E)-1-(2,4-dichlorophenyl)-4,4-dimethyl-2-(1,2,4-triazol-[^{14}C]-1-yl)-1-penten-3-one (7)(26.7 mCi, 337 mg, 1.04 mmole) in methanol (8.5 ml). The mixture was stirred at 0-5 °C for 1.5 hr. After decomposition with water, and hydrochloric acid, the mixture was extracted with ether. The extract was washed with water, dried, and evaporated to give a residue (26.2 mCi), as a 9:1 mixture of 1 and 2. Chromatography of the residue on silica gel with benzene-ether (gradient) gave (E)-S-3308-(triazole-^{14}C)(1)(20.8 mCi, 264 mg, yield 78%, purity 99%); mp 148 °C; TLC: Rf(D) 0.36, Rf(F) 0.07, Rf(G) 0.55; GC_3: retention time 16.0 min; NMR (δ, $CDCl_3$): 0.70 (9H, s, tert-butyl H), 4.50 (2H, q. methine and hydroxyl H), 6.90 (1H, s, olefinic H), 7.20-7.60 (3H, m, aromatic H), 8.07 (1H, s, triazole H), 8.60 (1H, s, triazole H).

Optical resolution of (E)-S-3308-(triazole-^{14}C)(1) -- A solution of (E)-S-3308-(triazole-^{14}C)(1)(20.8 mCi, 264 mg) in dioxane (846 µl) was injected by portions (50 µl) on a HPLC system (column: Sumipax OA-2200 , 8 mm x 25 cm (x 2), mobile phase: n-hexane/1,2-dichloroethane/ethanol = 450/40/4, flow rate: 3.0 ml/min). Fractions containing the (-)-isomer (retention time 12.2 min) and the (+)-isomer (retention time 15.0 min) were collected, combined, and evaporated to afford (-)(E)-S-3308-(triazole-^{14}C)(S-3308 L-^{14}C)(1a)(9.9 mCi, 126 mg, specific activity 25.7 mCi/mmole, yield 95%, purity 99%) and (+)(E)-S-3308-(triazole-^{14}C)(1b)(10.4 mCi, 132 mg, specific activity 25.7 mCi/mmole, yield 100%, purity 99%), respectively. These products were identical in every respect with the unlabeled authentic samples.

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